EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST)*

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Abstract

The European Committee on Antimicrobial Susceptibility Testing-Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) has revised the EDef 7.1 document on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for fermentative yeasts. Changes are: dimethylsulphoxide is now the recommended solvent for caspofungin, micafungin and fluconazole; the shelf-life of plates containing the echinocandins prepared from stock solutions in dimethylsulphoxide is extended to 6 months at −80°C; testing of amphotericin and *Cryptococcus* has been incorporated; and minimum inhibitory concentration ranges for quality control strains and anidulafungin are included.

Keywords: antifungals, *Candida*, *Cryptococcus*, EUCAST, minimum inhibitory concentration, susceptibility testing, yeast

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This Technical Note is based on the EUCAST definitive document EDef 7.2 (available at http://www.eucast.org/antifungal_susceptibility_testing/methods_of_antifungal_susceptibility_testing/susceptibility_testing_of_yeasts/).

The EUCAST Definitive Document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts was approved and published as a discussion document (7.1) in 2003 [1] and the definitive document was published in 2008 [2].

The European Committee on Antimicrobial Susceptibility Testing–Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) has revised the document to reflect current knowledge regarding the testing of non-fermentative yeasts (most importantly *Cryptococcus*) and technical details regarding MIC determinations for echinocandins and amphotericin B. This Technical Note is based on the EUCAST definitive document EUCAST definitive document EDef 7.2: *Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts* (available on the EUCAST website: http://www.eucast.org). In the full document the revised method is described in detail.

Main updates are as follows. (i) Dimethylsulphoxide (DMSO) is recommended as solvent for fluconazole and all three echinocandins and shelf-life is extended to 6 months. The motivation is that the commercially available fluconazole pure substance and three echinocandins are hydrophobic, that considerable variation in MICs has been demonstrated when water is used as solvent, particularly for caspofungin, and that susceptibility plates are stable at −80°C for up to 6 months when DMSO is used [3]. (ii) For the time being it is recommended that the RPMI 2% glucose is used also for amphotericin B. The non-synthetic broth Antibiotic Medium 3 (AM3), supplemented to a final concentration of 2% glucose, has been evaluated for detecting resistance to amphotericin B [4–7]. However, there is batch-to-batch variation in this medium, and also in the performance of the medium from different manufacturers. (iii) Susceptibility testing of *Cryptococcus* has been included. Main recommendations for the time being are that the EUCAST methodology is adopted and the plates are read when the optical density value is >0.2. In cases with insufficient growth it is suggested that the test be repeated but that trays are incubated at 30°C [8].

(iv) The following quality control MIC ranges have been included for anidulafungin and *Candida krusei* ATCC 6258: MIC ≤0.06 mg/L and for *Candida parapsilosis* ATCC 22019: MIC range 0.25–1.0 mg/L.

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Transparency Declaration

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